IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	**Markus STORR et al.**		
Application No.:	**10/572,703**	Art Unit:	1797
Filed:	March 17, 2006	Examiner:	David C. MELLON
For:	Separating Material		

DECLARATION PURSUANT TO 37 CFR 1.132

I, Dr. Markus Storr, PhD., Dipl.-Chem. (Univ.) do hereby declare:

THAT, I am a German citizen, residing at Achterwaldstrasse 28, 70794 Filderstadt, Germany

THAT, I have been employed by Gambro Dialysatoren GmbH since 1994, and am currently a senior scientist and research project manager at Gambro Dialysatoren GmbH in Hechingen, Germany.

THAT, I have carried out research and development on synthetic membranes and dialyzers including the functionalization of membranes for different uses during the time of my employment with Gambro Dialysatoren GmbH.

THAT, I am familiar with the invention set forth in United States Patent Application No. 10/572,703 filed on March 17, 2006 entitled: "Separating Material."

THAT, I have supervised the following experiments directed to the processes for grafting of polymers involving amino functional groups:

Experiment Design:

We have performed tests to determine if the process suggested by Hörl et al. (US 5,556,708) for grafting monomers to a polymer exhibiting a primary amino group is a possible way to couple monomers to amino functional groups on a polymer in the absence or presence of any organic solvent.

To that end, we undertook to couple

(A) N,N-dimethylaminopropylacrylamide (DMPA) as disclosed in Storr et al. (US 10/572,703) to Toyopearl EP70 R 501 GC resin exhibiting primary amino groups, according

(1) to the method disclosed in Hörl et al., and
(2) to the method disclosed in Storr et al.,

in the absence of any organic solvent.

(B) Dimethylaminopropylmethacrylamid (DMAPMA) as disclosed in Hörl et al. (US 5,556,708, col. 6, l.41f) to Toyopearl EP70 R 501 GC resin exhibiting primary amino groups, according

(1) to the method disclosed in Hörl et al., and
(2) to the method disclosed in Storr et al.,

in the absence of any organic solvent.

(C) N,N-dimethylaminopropylacrylamide (DMPA) as disclosed in Storr et al. (US 10/572,703) to Toyopearl EP70 R 501 GC resin exhibiting primary amino groups, according to the method disclosed in Hörl et al.,

in the presence of organic solvent, and

(D) Dimethylaminopropylmethacrylamid (DMAPMA) as disclosed in Hörl et al. (US 5,556,708, col. 6, l.41f) to Toyopearl EP70 R 501 GC resin exhibiting primary amino groups, according to the method disclosed in Hörl et al.,

in the presence of organic solvent (carbon tetrachloride) as disclosed in Hörl et al..

The monomers were chosen both from the disclosure of Storr et al. and Hörl. et al. in order to show that the monomer has no significant influence on the reaction (A) and (B).

Experiments (A) and (B) were carried out in the absence of any organic solvent, as our reaction is designed to work in water. In addition, we carried out the experiments (A1) and (B1) in the presence of an organic solvent (CCl_4) in order to clarify if the reaction described in Hörl et al. functions in the presence of the solvent.

The methods used and the results obtained are described below in more detail.

Methods:

1. Coupling of DMPA or DMAPMA to amino-functional groups according to Storr et al.

Aminated Toyopearl EP70 R 501 GC resin was used as starting material for all grafting reactions. Amination was done according to what has been described in Storr et al. (see Ex. 1).

(A) Coupling of the initiator

1.6% N-hydroxysuccinimide (NHS) was dissolved at room temperature in 75 ml of 0.1 M NaOH. Then, 1.1% azobis(4-cyanovaleric acid) was added and dissolved at room temperature. In parallel, 1.6% 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide · HCl was added to 5 ml of 0.1 M NaOH. Both solutions were pooled.

10g of the aminated beads were added to the solution and left at room temperature for a bit more than 16 hours. Afterwards, the beads were rinsed with 0.1 M NaOH and water.

(B) Coupling of the monomer

5.6 g of the respective monomer were added to 74.4g degassed water. The monomer was allowed to dissolve at room temperature and stripped with N_2 for one hour. The monomer solution was then added to the beads and a vacuum (<30mbar) was applied for 15 minutes. Afterwards, the reaction vessel was flooded with N_2 and the solution was again stripped for 15 minutes. In total, four cycles of vacuum, N_2 flooding and stripping were performed before the solution was heated to 75°C. An overpressure of about 50 mbar was applied to the reaction vessel. The reaction was allowed to proceed for about 16 hours under continuous stripping of the solution with N_2.

Finally, the beads were rinsed, in this order, with water, 1M HCl, 1M NaOH, 1M NaCl and water.

2. Coupling of DMPA or DMAPMA to amino-functional groups according to Hörl et al.

(A) Coupling of monomers (with CCl_4)

72g of phosphate buffer pH 8.0 were added to 8g of the monomer (10%). The monomer was allowed to dissolve at room temperature. The monomer solution was then transferred to the reaction vessel. 1% CCl_4 was added as well as 10g of the beads. A gold electrode was inserted for determining the redox potential in the reaction solution. The redox potential was adjusted to -340 mV to -440 mV with $Na_2S_2O_4$ (0.1%) and kept in that range by adding further $Na_2S_2O_4$ if needed. The reaction was allowed to proceed for 3 hours. Finally, the beads were rinsed with water.

(B) Coupling of monomers (without CCl_4)

72g of phosphate buffer pH 8.0 were added to 8g of the monomer (10%). The monomer was allowed to dissolve at room temperature. The monomer solution was then transferred to the reaction vessel. 10g of the beads were added to the solution. A gold electrode was inserted for determining the redox potential in the reaction solution. The redox potential was adjusted to -340 mV to -440 mV with $Na_2S_2O_4$ (0.1%) and kept in that range by adding further $Na_2S_2O_4$ if needed. The reaction was allowed to proceed for 3 hours. Finally, the beads were rinsed with water.

3. Determination of the grafting yield and protein binding capacity

The dynamic protein binding capacity of the products produced in the above-mentioned tests were determined by establishing the breakthrough curves of the fixed bed columns filled with the grafted Tosoh beads in single-pass perfusion mode.

BSA was exemplarily used. The BSA solution was prepared by adding 500 ml Tris buffer pH 8.0 (12.1 g Tris (pH 8.0) in 2 l water) to 1g BSA. After the BSA had dissolved, the solution was filled up to 1 l.

The grafted beads were dried under vacuum at a temperature of 40°C for 24 hours. 20 ml of the Tris buffer pH 8.0 were added to 1 g of the dries beads and left to swell for 15 minutes. The swelled beads were degassed under vacuum for about 30 minutes.

The UV detector used was a K-2001 (Knauer, Germany). The wavelength was adjusted to 280.

The beads were carefully transferred into a column in order to avoid the formation of air bubbles. The column was connected to the detector and the pump was started (1ml/min). Before the BSA solution was added, the system was run with the Tris buffer (pH 8.0) until a constant value was achieved (base line).

During the measurement the potential increases when BSA is detected at the chosen wavelength. As soon as a constant value was reached, no additional BSA is adsorbed to the matrix. Accordingly, the measurement was then completed and the results could be analysed.

The adsorption capacity for BSA [mg/g] was calculated as follows:

$$\text{weight } BSA_{adsorbed}/\text{weight Beads} = (\text{weight}_{afflux}-\text{weight}_{outflow})/\text{weight Beads [mg/g]}$$

Untreated, aminated beads (starting material) were also submitted to the BSA binding test as described in order to determine the monomer-independent adsorption to the matrix.

Results:

The results of the reactions carried out according to the above methods are summarized in **Table I.**

We have tested the coupling efficiency of two different monomers to beads exhibiting primary amino groups by determining the BSA adsorption capacity of the resulting matrices. We have

used two different approaches, i.e. the process as described in Hörl et al. and the process as described in Storr et al.

We have found that the BSA adsorption to beads which have been modified according to Hörl et al. is very low. The amount of BSA adsorbed in these cases is in the range of the adsorption of BSA to untreated beads. The reaction according to Hörl et al. is somewhat improved in the presence of an organic solvent, but the amount of BSA adsorbed is still not significant compared to untreated beads. The monomers chosen here do not have a significant influence on the adsorption ability of the matrix.

	Method	Exp. ID	Mono-mer	Mono-mer conc. [%]	CCl_4 conc. [%]	$Na_2S_2O_4$ conc. [%]	Redox Potential [mV]	Reaction Time [hours]	BSA Adsorption [mg/g_{Beads}]
1	Hörl et al.	A1	DMPA	10	-	5	> -340/-440	3	34
2	Hörl et al.	A1	DMPA	10	-	0.1	-340/-440	3	41
3	Storr et al.	A2	DMPA	7	-	-	-	>16	355
4	Hörl et al.	B1	DMAPMA	10	-	0.1	-340/-440	3	39
5	Storr et al.	B2	DMAPMA	5	-	-	-	>16	288
6	Storr et al.	B2	DMAPMA	10	-	-	-	>16	379
7	Hörl et al.	C	DMPA	10	1	0.1	-340/-440	3	64
8	Hörl et al.	D	DMAPMA	10	1	0.1	-340/-440	3	46
9	untreated Material	-	-	-	-	-	-	-	19

Table I

The beads which have been modified according to Storr et al. show a significant adsorption of BSA, irrespective of the monomer used. In contrast, a significantly higher BSA adsorption is reached also when using a lower monomer concentration (7 or 5% compared to 10% for Hörl et al.). Again, the monomers used here have has no significant influence on the adsorption ability of the matrix.

The adsorption of BSA can also be visualized in form of curves which are plotted during the scanning process as described above under section 3 of "Methods" (**Fig. 1**) and correspond to the experiments shown in Table I (-●- (9); -♦- (3); -◇- (8); -■- (6); -○- (2); -□- (7); -▲-(4)). The potential [mV] reflects the BSA which leaves the column. As can be seen, virtually no BSA is adsorbed to the beads which were treated according to the method of Hörl et al. The potential quickly reaches a plateau, reflecting the lack of adsorption to the beads. BSA is adsorbed to the

untreated aminated starting material in about the same range as to the matrix obtain by the reaction according to Hörl et al.

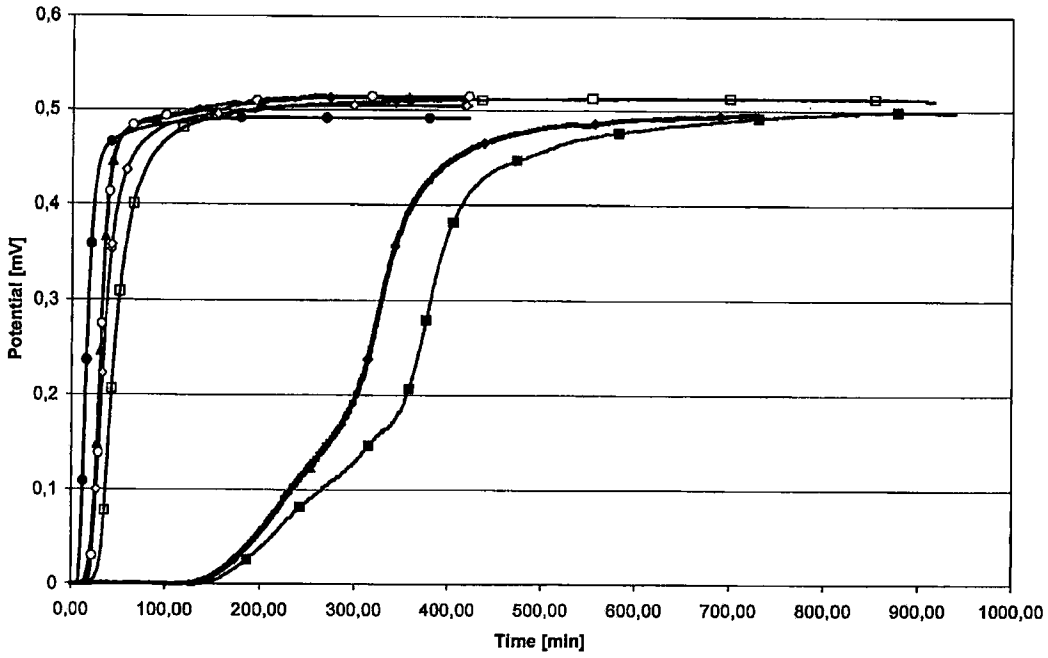

Figure 1

Conclusion:

As a result, we conclude that BSA is not adsorbed to the matrix as prepared according to Hörl et al. in an amount which is significantly higher than it is adsorbed to an untreated matrix having no monomers grafted onto it. This result is not due to the monomer chosen. We also conclude that the presence of CCl_4 only insignificantly improves the coupling of the monomers to the primary amino groups.

Therefore, we conclude that the method disclosed in Hörl et al. is not applicable for the grafting of monomers to primary, and as a consequence also to secondary, amino groups for obtaining a separation material. The process will not result in an utilizable separating material such as can be obtained by the method disclosed by Storr et al.

I hereby declare that all the statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements are made with the knowledge that willful false statements are so made punishable by fine or imprisonment, or both, under Section 101 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

28.7.2010
Date

Markus STORR